STUDIES ON ADRENAL HOMOGENATES

A. CHOLESTEROL CONVERSION

B. PEROXIDATION IN ADRENAL METABOLISM

BY

BERNARD DUBNICK

B. S., The City College of New York

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SUBMITTED IN PARTIAL FULFILLMENT of the REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN
CHEMISTRY IN THE GRADUATE COLLEGE
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URBANA, ILLINOIS

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ABSTRACT OF THESIS

INTRODUCTION

Our attempt was to relate metabolic events as observed by manometry to steroid conversions in the adrenal gland.

A previous communication from this laboratory (1) reported that about 30% reduction in cholesterol occurs when whole guinea pig adrenal homogenates reinforced with ATP, DPN, nicotinamide and cytochrome c are incubated at pH 7.4 under oxygen. We extended the study of this system to discover the effect of pH changes and various additions to the incubation mixture upon respiration and cholesterol disappearance at this early stage in adrenal steroidogenesis.

The possibility that a peroxidase might be demonstrable in adrenal tissue and might be involved in steroid conversions was considered and tested.

The precise role which fumarate plays in 11B-hydroxylation is currently under examination by workers interested in that steroid conversion. It appears to be quite a specific requirement for the reaction in aged adrenal particulate preparations (2). Hayano and Dorfman (2) found that their stored tissue required ATP, DPN and magnesiumions in addition to fumarate. But TPN is reporated to replace the triple requirement (3). We have studied the oxidation of fumarate by aged beef adrenal tissue manometically to gain information about the nature of the fumarate involvement.

DISCUSSION OF RESULTS

We were able to show that cholesterol disappears at pH 6.0 as well as at pH 7.4, but not appreciably at pH 6.7 in 2 minute water homogenates (fortified as previously) of fresh whole guinea pig adrenals. Respiration at 28 degrees Centigrade was also found to be at least as good at pH 6.0 as at pH 7.4. Cholesterol disappearance was less with a homogenization time shorter than the usual 2 minutes. A balance of cholesterol disappearance and resynthesis was suggested, synthesis being encouraged by more integrated tissue and by a pH close to 6.7 (4, 5). However,

it is possible that the cholesterol disappearances at pH 6.0 and 7.4 occur via different routes. The effect of certain additions to the incubation medium was studied. Fumarate appeared to exert a small enhancing effect on cholesterol disappearance at pH 7.4. Antimycin A failed to inhibit the cholesterol disappearance at pH 6.0. And ACTH appeared to inhibit cholesterol disappearance at pH 6.0 while not affecting it at pH 7.4. Evidence was also presented suggesting that 10-15 per cent of adrenal sterols are of the "fast reacting" (6) type.

Beef adrenal cortex homogenate gives a positive benzidine peroxidase test which does not appear to be due to its blood or cytochrome c content. If beef adrenal homogenate is forced to oxidize succinate through methylene blue as hydrogen carrier by poisoning the tissue with antimycin A, an antibiotic which inactivates the Slater factor in the normal cytochrome-linked succinoxidase system (7), the tissue can apparently utilize the hydrogen peroxide produced in the reoxidation of leucomethylene blue in coupled peroxidation of ethyl alcohol, as observed by manometry. However, a coupled peroxidation of compound S could not be demonstrated satisfactorily by this means.

Beef adrenals frozen on dry ice upon collection and then stored at -12 degrees Centigrade for one month lost their ability to oxidize fumarate, although they still retained their succinoxidase activity. Fumaric acid oxidation was restored by the addition of both ATP and DPN or of TPN alone, which parallels the results of Hayano and Dorfman. Although succinoxidase activity of aged adrenals was susceptible to inhibition by antimycin A, the reoxidation of TPNH, either in fumarate or glucose-6-phosphate oxidation observed manometrically, was not affected by that antibiotic. The important role of TPN in adrenal metabolism, which has been the subject of investigation in these laboratories (8, 9) was reemphasized.

BIBLIOGRAPHY

1. Vestling, C. S. and Lata, G. F., Science **113** 582 (1951)

2. Hayano, M. and Dorfman, R. I., J. Biol. Chem., **201** 175 (1953)

3. Hayano, M., Wiener, M. and Lindberg, M. C., Fed. Proc., **12** 216 (1953)

4. Bucher, N.L.R.; McGovern, N.H.; Kingston, R. and Kennedy, M. H., Fed. Proc., **12** 184 (1953)

5. Curran, G. L., J. Biol. Chem., **200** 17 (1953)

6. Moore, P. R., and Baumann, C. A., J, Biol. Chem, **195** 615 (1952)

7. Potter, V. R. and Reif, A. E., J. Biol. Chem., **194** 287(1952)

8. Nielson, E. D., "Enzymatic Studies of Adrenal Tissue," Thesis for Ph.D. in Chemistry, University of Illinois (1948)

9. Kelley, T. L., "Studies on Glucose-6-Phosphate Dehydrogenase of Adrenal Tissue, "Thesis for Ph. D. in Chemistry, University of Illinois (1953)

VITA

The author was born on May 29, 1928, in Brooklyn, New York, and attended parochial elementary school and public high school in that city. Upon graduation from Thomas Jefferson High School he enrolled in The City College of New York in 1945, under a New York State Scholarship and received his B. S. from that institution in June, 1949. He entered the Graduate School of the University of Illinois in September, 1949. He held a research assistantship sponsored by the Upjohn Company from September, 1950, through August, 1953, and a quarter time teaching assistantship in Inorganic chemistry for two semesters in 1952-53.

The author was married to the former Maxine Audrey France in August, 1952.